# Interleukin 18-independent engagement of interleukin 18 receptor-alpha is required for autoimmune inflammation.

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Abstract: T helper type 1 (T(H)1) lymphocytes are considered to be the main pathogenic cell type responsible for organ-specific autoimmune inflammation. As interleukin 18 (IL-18) is a cofactor with IL-12 in promoting T(H)1 cell development, we examined the function of IL-18 and its receptor, IL-18R, in autoimmune central nervous system inflammation. Similar to IL-12-deficient mice, IL-18-deficient mice were susceptible to experimental autoimmune encephalomyelitis. In contrast, IL-18R alpha-deficient mice were resistant to experimental autoimmune encephalomyelitis, indicating involvement of an IL-18R alpha ligand other than IL-18 with encephalitogenic properties. Moreover, engagement of IL-18R alpha on antigen-presenting cells was required for the generation of pathogenic IL-17-producing T helper cells. Thus, IL-18 and T(H)1 cells are dispensable, whereas IL-18R alpha and IL-17-producing T helper cells are required, for autoimmune central nervous system inflammation.

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# IL-18-independent IL-18Rα engagement is required for the development of autoimmune inflammation

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**$T_H1$ cells have long been thought to be the major pathogenic cell population in organ-specific autoimmune inflammation. As IL-18 is a vital cofactor, together with IL-12, in promoting $T_H1$ development, we examined the role of IL-18 and its receptor in the context of autoimmune CNS-inflammation. As with IL-12-deficient mice, we found that IL-18-deficient mice are fully susceptible to EAE. Surprisingly, we discovered that mice deficient in IL-18Rα are EAE-resistant, suggesting the presence of an alternative ligand with encephalitogenic properties. Our results establish that IL-18 and $T_H1$ development are dispensable for autoimmune inflammation, while IL-18Rα engagement on APCs is essential for the generation of encephalitogenic $T_H17$ cells which are now considered to be the major pathogenic population in autoimmune diseases.**

Tissue-specific, cell-mediated autoimmune diseases, such as rheumatoid arthritis (RA), type I diabetes or multiple sclerosis (MS), are widely held to be mediated by autoreactive $T_H$ lymphocytes. Until recently, MS and RA were firmly recognized to be $T_H1$-mediated, a notion supported by the findings obtained using their respective animal models, experimental autoimmune encephalomyelitis (EAE) and collagen-induced arthritis (CIA)[1, 2]. In EAE, for example, disease can be induced in susceptible rodents by adoptive transfer of myelin-reactive $T_H1$ cells as well as by active immunization with myelin antigens. Nevertheless, the main evidence supporting that pathogenic autoimmune cells are polarized $T_H1$ cells is based on the fact that tissue-invading T cells usually express IFN-γ[3, 4]. Furthermore, auto-aggressive $T_H2$ cells were believed to have anti-encephalitogenic properties and immune deviation is considered an attractive therapeutic strategy[5-7]. However, a paradigm shift was initiated by a number of important key findings: i) IFN-γ- as well as TNF-α-deficient mice were found to be EAE susceptible[8-11]. In fact, IFN-γ-deficiency even renders EAE-resistant mouse strains susceptible to EAE[9]. ii) while the p40 subunit of IL-12 was found to be critical for the development of autoimmunity, its binding partner p35 was shown to be irrelevant[12]. This phenomenon is explained by the fact that p40 is also the large subunit of IL-23, which, in contrast to IL-12, is vital for autoimmunity[12]. iii) IL-23 is, compared to IL-12, not a major $T_H1$ inducing cytokine but drives the expansion of IL-17-polarized T cells. IL-17, in contrast to IFN-γ, is indeed firmly linked with encepahlopathogenicity[13, 14]. Since IL-12, which was for many years believed to be the main pathogenicity-promoting factor in EAE, is no longer considered to be essential, we wanted to assess the involvement of IL-18 on $T_H1$ polarization and EAE development.

Interleukin (IL)-18 is a proinflammatory cytokine and a member of the IL-1 superfamily of cytokines. Its major function during inflammation appears to be associated with the polarization of $T_H$ cells[15-17]. IL-18 is a vital cofactor, together with IL-12, for the generation of IFN-γ-

secreting $T_H1$ cells. It is secreted by APCs and signals via the IL-18R, a heterodimer consisting of a ligand-binding IL-18Rα-subunit and a signaling IL-18Rβ-subunit (IL-1RAcPL; IL-1R7). Downstream signaling of the IL-18R, like that of other IL-1Rs and TLRs, activates IRAK4 and MyD88[18-20]. IL-18R is expressed on lymphocytes and has more recently been found to be expressed on accessory cells[21-24]. While it is firmly established that IL-18 can bind to the IL-18R complex, its affinity to IL-18Rα is only weak[25, 26]. The fact that IL-$18^{-/-}$ mice have been demonstrated to be EAE-resistant[27], causes a dilemma given the clearly protective activity of IL-12[12, 28, 29] and IFN-γ[30]. Therefore, we sought to identify the mechanism by which IL-18 drives autoimmunity.

Here we show that IL-18 does not exert a visible pathogenic effect in EAE and IL-$18^{-/-}$ mice are fully susceptible to EAE. However, deletion of its proposed receptor (IL-18Rα) results in complete resistance to EAE, suggesting the presence of an alternative ligand with encephalitogenic properties. Loss of IL-18Rα affects neither the priming nor expansion of Ag-driven T cells. However, engagement of IL-18Rα on APCs is critical for the generation of $T_H17$ cells in an IL-23-dependent fashion.

## Results

### Mice deficient in both IL-12p35 and IL-18 are fully susceptible to EAE

EAE is generally referred to as a $T_H1$-mediated disease yet deletion of IL-12p35, a major $T_H1$-promoting cytokine, renders mice susceptible to MOG-peptide-induced EAE[12]. Shi et al. have demonstrated, however, that mice deficient in IL-18, a cytokine that synergizes with IL-12 in $T_H1$ polarization, are resistant to EAE[27]. To assess whether IL-18 is capable of compensating for the loss of IL-12 in $p35^{-/-}$ mice, rendering them EAE susceptibility, we generated mice deficient in both IL-12p35 and IL-18 ($p35^{-/-}$x $IL\text{-}18^{-/-}$). Immunization with $MOG_{35\text{-}55}$ emulsified in CFA showed that $p35^{-/-}$x $IL\text{-}18^{-/-}$ mice are fully susceptible to EAE and have a similar disease severity and development as is seen in wt mice (**Fig. 1a and Table 1**). This demonstrates that IL-18 is not responsible for the EAE susceptibility of $IL\text{-}12p35^{-/-}$ mice and implies that IL-18 itself is a cytokine that has little or no effect on EAE pathogenesis. To confirm this, we actively immunized wt and single-mutant $IL\text{-}18^{-/-}$ mice with MOG peptide and found that $IL\text{-}18^{-/-}$ mice are indeed fully susceptible to EAE (**Fig. 1b and Table 2)**.

### IL-18 is required for mitogen- but not Ag-driven immunity

In order to rule out the possible expression of a truncated yet functional form of IL-18, we extensively verified the targeting strategy and genotype of the mice. We could clearly establish that $IL\text{-}18^{-/-}$ mice do not produce IL-18 mRNA by performing RT-PCR with primers located outside the target of deletion. We also analyzed by ELISA whether we could detect IL-18 secreted from activated splenocytes derived from wt and $IL\text{-}18^{-/-}$ mice, which showed that $IL\text{-}18^{-/-}$ mice are indeed completely IL-18 deficient (**Supplementary Fig. 1** online). As observed in many experimental systems, the deletion of IL-18 consistently results in the paucity of an IFN-γ response[31, 32]. We thus stimulated lymphocytes derived from naïve wt, $IL\text{-}18^{-/-}$ and, in addition,

IL-18Rα$^{-/-}$ mice *in vitro* with the lectin Concanavalin A (ConA) for 16 hours and IFN-γ production was subsequently measured by ELISA. Consistent with the principle that IL-18 has an effect on IFN-γ production, lymphocytes from both IL-18$^{-/-}$ and IL-18Rα$^{-/-}$ mice did not secrete IFN-γ in contrast to wt lymphocytes (**Fig. 2a)**. We could further verify that T cell function and activation during polyclonal stimulation is not directly impaired as there was no difference in IFN-γ secretion by stimulated wt, IL-18$^{-/-}$ and IL-18Rα$^{-/-}$ purified CD4$^{+}$ T cells (**Supplementary Fig. 2** online).

To establish the role of IL-18 and IL-18Rα in an adaptive Ag-driven immune response, we immunized wt, IL-18$^{-/-}$ and IL-18Rα$^{-/-}$ mice subcutaneously with $MOG_{35-55}$ or KLH (as a large immunogenic protein Ag ≈ 400 kDa) in CFA and 7 days later isolated and restimulated lymphocytes with the cognate Ag *in vitro*. Surprisingly, when KLH was used, we did not observe a drastic difference in the IFN-γ-producing ability of lymphocytes derived from wt, IL-18$^{-/-}$ and IL-18Rα$^{-/-}$ mice (**Fig. 2b**). The less immunogenic MOG-peptide, however, revealed that IL-18Rα$^{-/-}$ mice produced significantly less IFN-γ than wt or IL-18$^{-/-}$ mice. Our data support the notion that while IL-18 and IL-18Rs are critical co-factors for the early IFN-γ response of mitogen- or lectin-activated T cells, peptide-induced responses revealed discordant behavior between lymphocytes lacking IL-18 or IL-18Rα. On the other hand, activation and IFN-γ production through a complex large protein Ag appears to be independent of IL-18 or IL-18Rα, a concept that is in agreement with the recent report by Santos et al[33].

**IL-18Rα$^{-/-}$ and anti-IL-18Rα mAb-treated mice are resistant to EAE**

Mice deficient in IL-18Rα have been described as having an immunological phenotype similar to that of IL-18$^{-/-}$ mice[34]. Given the discordant behavior of IL-18- and IL-18Rα-deficient lymphocytes towards MOG-peptide in recall-responses, we discovered that IL-18Rα$^{-/-}$ mice were,

in sharp contrast to both wt and IL-18$^{-/-}$ mice, resistant to EAE induction (**Fig. 3a** and **Table 2**). Histological analysis of spinal cords obtained 28 days after EAE induction showed that EAE-susceptible wt and IL-18$^{-/-}$ mice incur significant inflammation and demyelination as revealed by H&E and LFB staining, respectively (**Fig. 4**). A more detailed analysis demonstrated the infiltration of T cells, macrophages and B cells and axonal damage in the spinal cords of these mice (**Fig. 4** and **Supplementary Fig. 3a** online). In contrast, IL-18Rα$^{-/-}$ samples demonstrated no leukocyte infiltration or demyelination (**Fig. 4 and Supplementary Fig. 3a** online). Quantitative RNA analysis of spinal cords from mice with EAE showed that in accordance with the presence of inflammatory infiltrates, wt and IL-18$^{-/-}$ mice had increased expression of chemokines and inflammatory cytokines while these were decreased or absent in IL-18Rα$^{-/-}$ mice (**Supplementary Fig. 3b** online).

The discordant behavior of IL-18$^{-/-}$ and IL-18Rα$^{-/-}$ mice with regards to EAE strongly points towards an additional IL-18Rα ligand with powerful encephalitogenic properties. In order to verify that IL-18Rα and IL-18 have independent biological functions, we blocked IL-18Rα in EAE-susceptible IL-18$^{-/-}$ mice. Treatment of IL-18$^{-/-}$ mice with anti-IL-18Rα mAbs, given 1 day pre-immunization and every 3 days thereafter until the end of the experiment, significantly reduced disease development (**Fig. 3b**). Administration of anti-IL-18Rα mAbs did not lead to deletion of IL-18Rα-expressing cells nor did it alter the composition of peripheral leukocytes in the blood, LN or spleen (**Supplementary Table 1** online). Interestingly, treating IL-18$^{-/-}$ mice with mAbs against IL-18Rα post-immunization (day 10 p.i.) also abrogated EAE progression (**Fig. 3c**) suggesting that IL-18Rα engagement is an important event during the effector phase of EAE. Combining the facts that IL-18Rα blockade prevents EAE even in mice in which its ligand is completely removed by gene-targeting and that IL-18 has a reportedly low affinity to IL-18Rα,

we propose that another ligand must be responsible for the engagement, signaling and immune development mediated by IL-18Rα.

**IL-18Rα engagement is required for the persistence of inflammatory cells during the effector phase of disease and $T_H17$ polarization**

EAE is characterized by a massive influx of inflammatory cells into the CNS at the peak of disease yet immune cells also invade the CNS prior to the onset of clinical symptoms[35, 36]. For example, recruitment of $CD4^+$ T cells into the CNS is critical for the initiation of the effector phase of EAE while invasion of polymorphonuclear leukocytes appears to have a role in orchestrating these events[37]. Therefore, to establish the impact of IL-18Rα on the capacity of inflammatory cells to invade the CNS at time-points of pre-clinical disease, we immunized mice and analyzed the CNS for inflammatory infiltrates on day 7 post-immunization by flow cytometry. In contrast to peak disease when the IL-18R$\alpha^{-/-}$ CNS is devoid of inflammatory infiltrates (**Fig. 4**), IL-18R$\alpha^{-/-}$ leukocytes (CD45-high cells) were capable of CNS infiltration to the same extent as those of wt and IL-$18^{-/-}$ mice at stages of pre-clinical disease (**Fig. 5a**). Detailed analysis of the invading cells revealed that in wt, IL-$18^{-/-}$ and IL-18R$\alpha^{-/-}$ CNS comparable numbers of $CD4^+$ T cells, granulocytes, macrophages and B cells were present (**Supplementary Fig. 3c**). Thus, IL-18R$\alpha^{-/-}$ inflammatory cells appear to be incapable of persisting or of maintaining CNS invasion during the effector phase of the disease. Interestingly, these results resemble data obtained in IL-$23^{-/-}$ mice, which are also resistant to $MOG_{35-55}$-induced EAE and in which the deficiency does not prevent infiltration of inflammatory cells into the CNS, as observed post-immunization but pre-disease onset[13].

The similarities between IL-18R$\alpha^{-/-}$ and IL-$23^{-/-}$ mice regarding their EAE resistance with concomitant inflammatory cell invasion into the CNS, provoked us to assess the impact of IL-

18Rα on IL-17 production. IL-17 producing $T_H$ cells ($T_H17$) are now widely held to be the main pathogenic population during autoimmune inflammation[13, 14]. Therefore, we quantified the number of IL-17 producing MOG-reactive T cells invading the CNS pre and post-clinical disease onset in mice immunized with $MOG_{35-55}$. ELIspot analysis revealed that immediately prior to disease onset (9dpi), the number of MOG-specific IFN-γ-secreting cells invading the CNS is similar between EAE susceptible IL-$18^{-/-}$ and resistant IL-$18R\alpha^{-/-}$ mice, whereas $T_H17$ cells are virtually absent from the CNS of IL-$18R\alpha^{-/-}$ mice (**Fig. 5b**). After disease onset, we could detect increased numbers of IFN-γ and IL-17-secreting cells from the susceptible IL $18^{-/-}$ and wt mice, whereas they were virtually absent from the CNS of immunized IL-$18R\alpha^{-/-}$ mice (**Fig. 5c**).

To determine whether IL-$18R\alpha^{-/-}$ mice are defective in generating IL-17-producing T cells, we analyzed IL-17 expression by freshly primed lymphocytes *in vitro*. We immunized wt, IL-$18^{-/-}$ and IL-$18R\alpha^{-/-}$ mice with either $MOG_{35-55}$ peptide or KLH and harvested lymphocytes from the draining LNs after 7 days. We performed real-time PCR analysis of mRNA obtained from these lymphocytes upon restimulation with their cognate Ag and showed that the expression of IL-17 mRNA is significantly decreased in IL-$18R\alpha^{-/-}$ cells in comparison to wt and IL-$18^{-/-}$ cells (**Fig. 6a**). We could corroborate this by ELISA using the cell culture medium of lymphocytes rechallenged with their cognate Ag in vitro, which demonstrated that IL-17 production is significantly decreased in IL-$18R\alpha^{-/-}$ but not IL-$18^{-/-}$ or wt lymphocytes (**Fig. 6b**). In contrast to the production of IFN-γ, which shows some dependence on the immunogenicity of the Ag, IL-17-secretion was consistently decreased even when KLH was used as the driving Ag. Thus, resistance of IL-$18R\alpha^{-/-}$ mice to EAE results apparently from their inability to generate $T_H17$ cells.

**Expression of IL-18Rα on accessory cells is essential for encephalitogenicity**

The lack of IL-18Rα completely prevents the development of EAE and $T_H17$ polarization, whereas its putative ligand IL-18 appears to be irrelevant. The cell type on which the IL-18Rα exerts its primary effects remains unknown. This is mainly due to the fact that IL-18Rs are expressed by various cell types and tissues[21, 27, 38]. However, one is likely to presume that the presence of IL-18Rα on $CD4^+$ T cells is critical for the subsequent polarization of $T_H17$ cells. In order to identify the cell and tissue location of the IL-18Rα lesion in EAE, we selectively expressed IL-18Rα on cells in the leukocyte compartment using irradiation bone-marrow (BM)-chimeras. Following irradiation and reconstitution, the immune compartment in secondary lymphoid tissues of recipient mice is comprised of hematopoietic cells derived from donor mice[39]. We generated BM-chimeras by transferring either a 4:1 ratio of $RAG^{-/-}$ and IL-$18R\alpha^{-/-}$ BM into wt recipients ($RAG^{-/-}$ + IL-$18R\alpha^{-/-}$ → wt) or IL-$18R\alpha^{-/-}$ BM only into wt recipients (IL-$18R\alpha^{-/-}$ → wt). Wt-BM was transferred into wt recipients as a control (wt → wt). $RAG^{-/-}$ mice do not have lymphocytes and the resulting chimera ($RAG^{-/-}$ + IL-$18R\alpha^{-/-}$ → wt) thus has an IL-18Rα-deficient lymphocyte compartment, whereas the majority of all other leukocytes has undisrupted IL-18Rα alleles.

As expected IL-$18R\alpha^{-/-}$ → wt mice were resistant to EAE upon immunization with MOG peptide (**Fig. 7a**). However, addition of BM from $RAG^{-/-}$ mice, which does not give rise to T or B cells and therefore expresses IL-18Rα only on accessory cells and not on lymphocytes, to IL-$18R\alpha^{-/-}$ BM was able to overcome the resistance of IL-$18R\alpha^{-/-}$ mice to EAE ($RAG^{-/-}$ + IL-$18R\alpha^{-/-}$ → wt) (**Fig. 7a**). Thus, IL-18Rα must exert its primary effects in the accessory cell (mono- and polymorphonucleated phagocytes, DCs & NK-cells) compartment. Again, this finding is unexpected, given that IL-18 is thought to exert its effects primarily on T cells and NK cells, but

it is completely consistent with the undisturbed T cell responses that we have thus far demonstrated (**Supplementary Fig. 2** online).

As IL-18Rα has been demonstrated to be present on myeloid cells[40], we decided to assess the capacity of IL-18Rα-deficient APCs to prime naïve T cells. To do so, we purified $CD4^+$ T cells from 2d2 mice that express a transgenic TcR specific for $MOG_{35-55}$ and co-cultured them with mature, MOG-peptide-pulsed wt, IL-$18^{-/-}$ and IL-$18R\alpha^{-/-}$ BM-derived DCs. Measurement of their proliferation by thymidine incorporation showed no difference in the stimulatory abilities of IL-$18R\alpha^{-/-}$ BM-DCs (**Fig. 7b**). To confirm this in an *in vivo* setting, we injected CFSE-labeled 2d2 cells into wt, IL-$18^{-/-}$ and IL-$18R\alpha^{-/-}$ mice, immunized them with $MOG_{35-55}$ and analyzed proliferation of 2d2 cells by flow cytometry after 4 days. Transferred 2d2 cells proliferated to the same extent in IL-$18R\alpha^{-/-}$ mice as in wt and IL-$18^{-/-}$ animals (**Fig. 7c**). Using adoptive transfer of TcR-Tg T cells, we were able to verify the notion that the IL-18Rα-deficiency lesions a non-lymphocytic leukocyte and through this, the capacity to polarize $T_H17$ cells (**Supplementary Fig. 4** online). With regards to the ability of myeloid cells to reach the CNS tissue, we generated mixed BM-chimeras of CD45-congenic wt and IL-$18R\alpha^{-/-}$ BM-donors into wt recipients and evaluated the capacity of myeloid populations to invade the CNS during disease. We confirmed that the mice carry a 1:1 ratio of wt and IL-$18R\alpha^{-/-}$ hematopoietic cells in the systemic compartment. Upon induction of EAE, both myeloid populations invade the CNS again at a 1:1 ratio, implicating that there is no migratory defect of myeloid cells (data not shown). Lastly, expression of activation markers and co-stimulatory molecules by LPS-matured DCs showed no difference in upregulation of CD80, CD86 and CD40 between wt, IL-$18^{-/-}$ and IL-$18R\alpha^{-/-}$ mice (data not shown).

To confirm the role and function of IL-18Rα signaling on accessory cells during the effector phase of EAE, we adoptively transferred encephalitogenic MOG-reactive T cells derived from wt

donor mice into both wt and IL-18Rα$^{-/-}$ recipient mice. Fully primed and activated encephalitogenic T cells derived from wt mice induced EAE in wt recipient mice, yet they were incapable of inducing clinical EAE in IL-18Rα-deficient hosts (**Fig. 7d**).

**IL-18Rα-deficient APCs show limited IL-12/23p40 secretion required for pathogenic $T_H17$ cell generation**

As restimulation with cognate Ag demonstrated a significant decrease in IL-17 production, we decided to analyze the capacity of APCs to secrete IL-12/23p40, which is necessary for the expansion of pathogenic $T_H17$ cells. To determine p40 secretion, we stimulated T cell-depleted splenocytes with αCD40 mAb for 36 hours before analyzing p40 production by ELISA. We found that in contrast to high levels of p40 production by wt and IL-18$^{-/-}$ APCs, IL-18Rα$^{-/-}$, similar to p40$^{-/-}$ APCs, showed impaired p40 production (**Fig. 8a**). Finally, we could validate the deficient IL-12/23p40 production in an adaptive immune response by restimulating LN preparations from MOG- and KLH-immunized mice in vitro and analyzing p40 production after 2 days. As expected, there was a significant decrease in the production of p40 by IL-18Rα$^{-/-}$ lymphocytes as opposed to wt and IL-18$^{-/-}$ lymphocytes (**Fig. 8b,c**). We failed to observe a concrete decrease in p19 expression by αCD40-stimulated DCs, indicating that the effect of IL-18Rα-deficiency on $T_H17$ cells is likely regulated at the level of p40 rather than p19. In addition, we did not observe a decrease TGFβ expression in IL-18Rα$^{-/-}$ APC's (data not shown). Our data indicate that APC activation results in the production of an IL-18Rα ligand driving IL-23 secretion by APCs. Therefore the resistance of IL-18Rα$^{-/-}$ mice to EAE seems to result from abrogated $T_H17$ cell development, which is likely the consequence of poor p40 production by IL-18Rα$^{-/-}$ APCs.

**Discussion**

Organ-specific inflammatory diseases generally result from inappropriate expansion and activation of effector T lymphocytes, which are capable of escaping peripheral tolerance and reacting vividly to self-antigens. Until recently, $T_H1$ lymphocytes were thought to represent the autoreactive T cells responsible for inducing cellular autoimmunity[3-7]. However, there is mounting evidence that $T_H17$ cells, and not $T_H1$ cells, are the pathogenic T cell effectors in autoimmunity and that their development is negatively regulated by both $T_H1$ and $T_H2$ cytokines[12-14, 28, 41-43].

Due to the plethora of data arguing against the $T_H1$ paradigm of autoimmunity, we were interested in characterizing the role of IL-18 in EAE. IL-18 synergizes with IL-12 in the differentiation of naïve $T_H$ cells into $T_H1$ cells. Therefore, our goal was to determine if the EAE susceptibility of IL-12$^{-/-}$ mice occurs as a result of redundancy, whereby the continued presence and activity of IL-18 in these mice is sufficient for EAE induction. Our data dismiss this hypothesis as IL-12p35$^{-/-}$x IL-18$^{-/-}$ double deficient mice are fully susceptible to EAE. On further analysis, we discovered that IL-18$^{-/-}$ mice are also fully susceptible to EAE. These data show that IL-18 does not compensate for the loss of IL-12, again supporting the notion that $T_H1$ cells and $T_H1$-inducing factors do not promote EAE development. These findings are not unexpected when taking into account the non-encephalitogenic effects of other $T_H1$ cytokines and the discovery of pathogenic $T_H17$ cells but they nevertheless contradict a previously published report[27]. We could rule out that the differential results were due to differences in reagents or alternative methods of immunization and most importantly the experiments were performed in the identical line of mice. Therefore we have so far been unable to elucidate the disparities between the two sets of results; yet in support of our findings, IL-18$^{-/-}$ mice have been shown to be fully susceptible to the development of experimental autoimmune uveitis (EAU)[44, 45]. Generally, the observations in

EAU with regards to gene-targeted mice match that of EAE. Additionally, a recent report directly supports our findings that IL-18 is unnecessary during Ag-induced $T_H1$ responses, while demonstrating that IL-18 is redundant in an Ag-induced arthritis model[33].

Despite the susceptibility of IL-18$^{-/-}$ mice, we discovered that IL-18Rα$^{-/-}$ mice are resistant to EAE, which implies the existence of an alternative IL-18Rα-binding ligand with encephalitogenic properties. As the affinity of IL-18 to IL-18Rα is fairly poor and requires heterotrimerization with IL-18Rβ for increased affinity[26, 46], the likelihood that there is another ligand with higher affinity for IL-18Rα is very high. There are a number of orphan receptors within the IL-1R superfamily and given the fact that these receptor subunits form heterodimers with one another[47], it is most likely that IL-18Rα not only has different binding partners, but also different ligands. We demonstrated the potency of this putative ligand by significantly attenuating disease development in IL-18$^{-/-}$ mice using anti-IL-18Rα antibodies. Given that the accepted IL-18Rα-ligand, IL-18, was not present in these mice and that their cellular constituents were not affected as a result of injecting the antibody, these results provide strong evidence for the existence of such an alternative IL-18Rα ligand and we are presently in the process of identifying this novel IL-18Rα-binding ligand as well as the composition of its receptor complex.

The development of EAE is dependent on the infiltration of activated CD4$^+$ T cells into the CNS, an event that is accompanied by the influx of other immune cells including B cells, macrophages and granulocytes[35-37]. Although inflammatory cells are clearly absent from the spinal cords of IL-18Rα$^{-/-}$ mice at the peak of clinical EAE, we could detect comparable CNS-invading leukocyte infiltration prior to the onset of disease. Therefore, the lack of IL-18Rα does not affect the migratory properties of these cells but their ability to persist within or maintain infiltration into the target organ. Interestingly, the presence of inflammatory infiltrates in the IL-18Rα$^{-/-}$ CNS without concomitant EAE susceptibility resembles the observations in IL-23$^{-/-}$

mice[48]. The resistance of IL-23$^{-/-}$ mice to EAE likely results from their inability to expand 17-producing $T_H$ cells[13]. $T_H$17 cells are a novel $T_H$ cell subset and while TGFβ1 seems to be necessary for their de novo differentiation[49-51], IL-23 has been suggested to be required for their survival and expansion[49]. We found that the MOG-reactive T cells invading the CNS of IL-18Rα$^{-/-}$ mice immediately prior to clinical disease development did not produce IL-17, when compared to wt or IL-18$^{-/-}$ mice. Comparable to IL-23-deficient mice, we have discovered that IL-18Rα$^{-/-}$ mice are deficient in producing IL-17 at both the RNA and protein levels.

Our goal was then to determine the mechanism of action of IL-18Rα activation and signaling during EAE, as the expression of IL-18Rα is broadly distributed[21, 27, 38]. As our previous experiments regarding T cell priming only focused on the development of $T_H$1 cells, it seemed likely that the lack of $T_H$17 cells resulted from the absence of IL-18Rα expression on this subpopulation of T cells. This was not the case, however, and we could demonstrate that the loss of IL-18Rα lesions an accessory cell without affecting the capacity of IL-18Rα-deficient $T_H$-cells to develop an encephalitogenic phenotype. Furthermore, the importance of IL-18Rα on an accessory cell was accentuated in an adoptive transfer experiment whereby encephalitogenic wt T cells could not induce EAE in IL-18Rα$^{-/-}$ mice. The overall capacity to activate T cells and their expansion is not affected by the loss of IL-18Rα. In addition, expansion of T cells is not affected by the loss of IL-18Rα from DCs and IL-18Rα-deficient APCs show no difference in their activation status.

Finally, we demonstrate that IL-18Rα signaling on APCs is critical for the secretion of IL-23p40 and the subsequent production of IL-17. Our data thus support the hypothesis that IL-17 secretion is dependent on the continuous support of $T_H$17-promoting APCs, as adoptive transfer of encephalitogenic lymphocytes into IL-18Rα$^{-/-}$ mice renders the cells incapable of causing

tissue damage. This also explains the ability of anti-IL-18Rα antibodies to prevent disease in IL-$18^{-/-}$ mice even after priming has occurred.

In summary, we provide additional evidence refuting the $T_H1$ paradigm of autoimmunity by demonstrating a non-pathogenic role for IL-18 in EAE. In contrast, however, the so-called IL-18Rα is critical for the development of EAE thus implying the presence of an alternative IL-18Rα-binding ligand. We show that IL-18Rα signaling on APCs is critical for IL-23p40 secretion and the ensuing development of encephalitogenic $T_H17$ cells thereby explaining the resistance of IL-$18R\alpha^{-/-}$ to $MOG_{35-55}$-induced EAE. The identification of the alternative IL-18Rα-binding ligand should provide a potent novel therapeutic approach for the treatment of organ-specific inflammatory diseases such as MS. The attractive feature of this IL-18Rα pathway for therapeutic targeting is that its loss does not completely suppress immunity, but rather specifically affects the behavior of pathogenic autoimmune effector T cells.

## Methods

**Mice:** Female C57BL/6 mice were purchased from Harlan Laboratories (Netherlands). IL-12p40$^{-/-}$ mice were purchased from Jackson Laboratories (USA). Homozygous IL-18$^{-/-}$ and IL-18R$\alpha^{-/-}$ (all backcrossed onto C57BL/6 for more than 12 generations) were provided by S. Akira (Osaka) and Rag1$^{-/-}$ mice were provided by R. Zinkernagel (Zurich) and were bred in house under specific pathogen free (spf) conditions. 2d2 (MOG-TcR-Tg) mice were provided by V. Kuchroo (Harvard Medical School, Boston, MA) Animal experiments and breeding were approved by the Swiss Veterinary Office (#69/2003 and #70/2003).

Irradiation Bone Marrow (BM)-chimeric mice were generated as described[52]. Briefly, BM-donor mice were euthanized using $CO_2$ and BM-cells were isolated by flushing femur, tibia, radius and hip bones with phosphate buffered solution (PBS). BM cells were then passed through a 100 μm cell strainer and cells were washed with PBS. Recipient mice were lethally irradiated with 1100 rads (split dose) and i.v. injected with 12-25x$10^6$ BM-cells. Engraftment took place over 8 weeks of recovery.

**Induction of EAE:** Mice were immunized subcutaneously with 200 μg of $MOG_{35-55}$ peptide (amino acid sequence: MEVGWYRSPFSRVVHLYRNGK), obtained from GenScript (Piscataway, NJ), emulsified in CFA (DIFCO, Detroit, MI). Mice received 200 ng pertussis toxin (PtX) (Sigma-Aldrich) intraperitoneally at the time of immunization and 48 hours later. For adoptive transfer, MOG-reactive T cells were generated as described[39]. Monoclonal α-IL-18Rα antibody (clone 112624) (R&D Systems) was administered either 1 day pre-immunization (450 μg/mouse) and every 3 days thereafter (300 μg/mouse) or every 3 days beginning from disease onset (300 μg/mouse). Mice were scored daily as follows: 0) no detectable signs of EAE; 0.5) distal tail limp; 1) complete tail limp; 2) unilateral partial hind limb paralysis; 2.5) bilateral partial

limb paralysis; 3) complete bilateral hind limb paralysis; 3.5) complete hind limb paralysis and unilateral forelimb paralysis; 4) total paralysis of fore and hind limbs (score > 4 to be euthanized); 5) death. Each time point shown is the average disease score of each group. Statistical significance was assessed using an unpaired Student's *t*-Test (∗ < 0.05; # < 0.01).

**Histology and flow cytometry:** Mice were euthanized with $CO_2$, followed by perfusion with PBS and subsequent perfusion with 4% paraformaldehyde (PFA) in PBS. The spinal column was removed and fixed in 4% PFA in PBS. The spinal cord was then dissected and paraffin-embedded prior to staining with either haematoxylin & eosin or CD3, B220 and MAC-3 antibodies (BD Pharmingen) to assess infiltration of inflammatory cells, luxol fast blue to determine the degree of demyelination or amyloid precursor protein to assess the extent of axonal damage.

For cytofluorometric analysis we used the following antibodies: αCD45, αCD4, αVα3.2, αCD11b, αGR1, and αB220 (BD Pharmingen). For analysis of CNS invading cells, mice were euthanized with $CO_2$ and perfused intracardially with PBS as described[52]. Spinal cord was flushed out with PBS and the brain was dissected to isolate the brainstem. Both tissues were homogenized and strained through a 100μm nylon filter (Fisher). After centrifugation the cell suspension was resuspended in 30% Percoll (Pharmacia) and centrifuged at 12,000 rpm for 30 min at 4°C. We collected the interphase cells and extensively washed them before staining. For flow cytometry, we incubated the antibody for 20 min at 4°C. We analyzed the cells using a FACSCalibur (BD Pharmingen) with CellQuest software. Post-acquisition analysis was performed using WinMDI 2.8 software (Scripps-Research Institute).

**Proliferation and cytokine assays:** Spleen, axillary and inguinal lymph nodes were isolated from naïve mice or mice primed by injections of 100 μg/flank of $MOG_{35-55}$ or KLH (Sigma)

emulsified in CFA 7 days earlier. $2x10^5$ cells were placed as triplicates in 96-well plates. For naïve cells, 5μg/ml ConA was used for stimulation for 16 hours and 5μg/ml αCD40 (FGK) was used for stimulation for 36 hours before analyzing cytokine production by ELISA. $CD4^+$ T cells were purified from naïve splenocytes using BD IMag Magnetic Beads (BD Pharmingen) and stimulated with 5 μg/ml αCD3 (2c11) and 5 μg/ml αCD28 (37N) for 36 hours before analyzing IFN-γ production by ELISA. $MOG_{35-55}$ or KLH reactive cells were stimulated in triplicate for 48 hours with either 50 μg/ml $MOG_{35-55}$ or KLH, 5 μg/ml ConA or medium and 0.5 μCi/ml $^3$[H]-thymidine was added after 24 hours to observe proliferative responses. Thymidine incorporation was assessed using a Filtermate Harvester and a scintillation and luminescence counter. For cytokine analysis, the culture supernatant of identical sister cultures was harvested after 48 hours and analyzed in duplicate for IFN-γ, IL-17 and IL-23p40 production by ELISA (Pharmingen, La Jolla, CA). For real-time PCR analysis, RNA was isolated from restimulated cells by TRIzol extraction (Invitrogen).

**ELIspot:** For ELIspot assays, lymphocytes were isolated from the CNS of $MOG_{35-55}$-immunized mice on days 9 and 14 dpi by differential percoll centrifugation as described above. $2 \times 10^5$ cells were plated in complete RPMI containing 50 μg/ml $MOG_{35-55}$ in 96-well plates (Millipore) coated with 7.5 μg/ml αIFN-γ (AN18; Mabtech) or 2 μg/ml αIL-17 (TC11-18H10; BD Pharmingen) Abs. The plates were incubated at 37°C, 5% $CO_2$ for 18 (IL-17) or 20 (IFN-γ) hours at which point cells were discarded and plates were washed with PBS. 0.5 μg/ml αIFN-γ-bio (R4-6A2; Mabtech) or 1 μg/ml αIL-17-bio (TC11-8H4.1; BD Pharmingen) Abs were added and incubated at RT for 2 and 4 hours, respectively. After washing, streptavidin-alkaline phosphatase (Mabtech) was added to the plate and incubated for 1 hour at RT. The plates were washed with PBS and 100 μl of the substrate solution BCIP/NBT-plus was added to the wells and developed until distinct spots emerged. The plates were analyzed using an elispot reader (CTL immunospot).

**Generation of BM-derived DCs:** BM-derived DCs were generated as described[53]. Briefly, BM-donor mice were euthanized using $CO_2$ and the femur and tibia were removed. BM-cells were isolated by flushing the bones with PBS and were filtered through a 100μm cell strainer. Cells (2-2.5x$10^6$ in 10 ml) were cultured in RMPI containing 10% FCS with the addition of 10% conditioned medium obtained from GM-CSF-transfected X-63 cells (obtained from A. Rollink, University of Basle, Switzerland). After at least 6 days, BM-derived DCs were matured with 1μg/ml LPS overnight while immature BM-derived DC's were maintained in GM-CSF-containing medium. BM-derived DCs were used from day 7 to 9.

**Tg T cell proliferation and polarization:** For *in vitro* proliferation of transgenic T cells, spleens were harvested from naïve 2d2 mice and $CD4^+$ T cells were purified using BD-IMag magnetic beads (BD Pharmingen). The purity of T cell isolation was verified by FACS analysis. 1x$10^5$ 2d2 T cells were cultured in a 96-well plate together with 3,000-10,000 immature or mature BM-DCs. Prior to co-culture, BM-DCs were pulsed with 1 μg/ml $MOG_{35-55}$ in RPMI for 3 hours, followed by washing and irradiation with 2000 rads. Non-pulsed DCs were used as a control as well as T cells cultured alone. Cells were incubated for 4 days and $^3$[H]-thymidine was added for the last 18 hours of culture.

For *in vivo* proliferation of TcR Tg cells, mice were injected i.v. with 25x$10^6$ 10μM CFSE (carbofluorescein diacetate succinimidyl ester) (Invitrogen-Molecular Probes)-labeled 2d2 cells and immunized s.c. by bilateral flank injection of 100μg $MOG_{35-55}$ emulsified in CFA. After 4 days, mice were killed and LN cells were isolated and stained with an antibody against the MOG-Tg TCR, αVα3.2, prior to cytofluorometric analysis.

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**Figure 1**

IL-18 is not required for EAE induction. EAE was induced by subcutaneous immunization with $MOG_{35-55}$ emulsified in CFA. Data display the mean EAE score of each group. (a) EAE progression in $p35^{-/-}$x $IL\text{-}18^{-/-}$ double deficient and wt mice. Shown is one representative of 2 experiments ($n \geq 5$ mice/group). (b) EAE progression in wt and $IL\text{-}18^{-/-}$ mice. Shown is one representative of 3 individual experiments.

**Figure 2**

IL-18 is required for mitogen-, but not Ag driven $T_H1$ development. (a) ELISA assessing IFN-γ secretion by naïve wt, $IL\text{-}18^{-/-}$ and $IL\text{-}18R\alpha^{-/-}$ LN cells, stimulated for 16 hours with 5 μg/ml ConA or medium. (b) ELISA of IFN-γ in supernatant from mice immunized with 200 μg $MOG_{35-55}$ or KLH in CFA. 7 days after immunization, LN cells were isolated and restimulated in duplicate with either 50 μg/ml $MOG_{35-55}$ or KLH and medium for 48 hours. Data are representative of at least 2 individual experiments and combine 2 mice in each group.

**Figure 3**

Discordant behavior of $IL\text{-}18^{-/-}$ and $IL\text{-}18R\alpha^{-/-}$ mice in EAE. EAE was induced by subcutaneous immunization with $MOG_{35-55}$. Data display the mean EAE score of each group. (a) EAE progression in wt, $IL\text{-}18^{-/-}$ and $IL\text{-}18R\alpha^{-/-}$ mice. Shown is one representative of 3 individual experiments. (b) $IL\text{-}18^{-/-}$ mice were treated with 450 μg α-IL-18Rα Ab or control IgG 1 day pre-immunization with $MOG_{35-55}$ and with 300 μg antibody for every 3 days thereafter. (c) $IL\text{-}18^{-/-}$ mice were immunized and treated with 300 μg α-IL-18Rα Ab or control IgG at the first sign of disease. Shown are representatives of at least two individual experiments ($n \geq 5$ mice/group).

**Figure 4**

Histopathological analysis of IL-18$^{-/-}$ and IL-18Rα$^{-/-}$ mice. Paraffin-embedded spinal cord sections from PBS-perfused wt, IL-18$^{-/-}$ and IL-18Rα$^{-/-}$ animals on day 28 after induction of EAE with $MOG_{35-55}$. Haematoxylin and eosin (H&E) assesses cell infiltration, luxol fast blue (LFB) assesses demyelination, MAC3$^{+}$, CD3$^{+}$ and B220 stainings demonstrate macrophage, T cell and B cell infiltration, respectively, and amyloid precursor protein (APP) assesses axonal damage. Scale bar = 100μm, scale bar for CD3 and B200 inserts = 50μm, scale bar for APP inserts = 25μm.

**Figure 5**

Tissue invasion at pre-clinical stages is not affected by IL-18Rα. Wt, IL-18$^{-/-}$ and IL-18Rα$^{-/-}$ were actively immunized with $MOG_{35-55}$ and at different time-points, the cerebelli and spinal cords were isolated. CNS mononucleated cells were isolated as described in M&M. (a) CNS-derived leukocytes were obtained from mice 7 dpi, stained with CD45 and CD11b and analyzed by flow cytometry. CD45$^{hi}$ cells represent cells invading the CNS, CD45$^{lo}$ cells represent CNS-residents. Shown is one representative of 3 individual experiments. (b,c) IFN-γ and IL-17 ELIspot analyses of CNS-derived MOG-reactive lymphocytes restimulated for 18-20 hours with $MOG_{35-55}$. Data in the bar graphs represent at least 2 individual experiments and combine 2 mice in each group. Shown is the mean ± S.E.M.

**Figure 6**

$T_H17$ induction is dependent on IL-18Rα but not IL-18. Wt, IL-18$^{-/-}$ and IL-18Rα$^{-/-}$ mice were immunized with $MOG_{35-55}$ or KLH in CFA and 7 days later, LN cells were isolated and restimulated for 48h with 50 μg/ml $MOG_{35-55}$ or KLH. Data are representative of 2 individual

experiments combining at least 2 mice per group. Shown is the mean ± S.E.M. (a) Real-time PCR analysis of IL-17 mRNA expression by wt, IL-18$^{-/-}$ and IL-18Rα$^{-/-}$ lymphocytes. Results are normalized to β-actin expression and analyzed in duplicate. (b) ELISA of IL-17 protein expression by $MOG_{35-55}$ and KLH-restimulated lymphocytes, analyzed in duplicate.

**Figure 7**

The absence of IL-18Rα specifically lesions accessory cells but not lymphocytes. (a) EAE progression in IL-18Rα$^{-/-}$ → wt, IL-18Rα$^{-/-}$ + RAG$^{-/-}$ → wt and wt → wt bone-marrow chimeric mice actively immunized with $MOG_{35-55}$ peptide. Shown is one representative of 2 individual experiments ($n \geq 5$ mice/group). (b) BM-derived DCs were generated from wt, IL-18$^{-/-}$ and IL18Rα$^{-/-}$ mice, matured with 10 μg/ml LPS and subsequently pulsed with 1 μg/ml MOG peptide. 2d2-specific CD4$^{+}$ T cells were purified from naïve 2d2 mice and co-cultured with the peptide-pulsed, irradiated DCs for 3 days. Proliferation was assessed by the incorporation of $^{3}$[H]-thymidine during the final 24 h in triplicate wells. (c) In vivo proliferation of 2d2-transferred cells into wt, IL-18$^{-/-}$ and IL-18Rα$^{-/-}$ mice. 25x10$^{6}$ CFSE-labeled 2d2 mice-derived splenocytes were injected i.v. into recipient mice, which were subsequently immunized with $MOG_{35-55}$ in CFA. 4 days later, LNs were isolated, cells were stained with Vα3.2 and the proliferation of 2d2 cells was analyzed by flow cytometry. (d) A polyclonal population of MOG-reactive lymphocytes was generated as described[52]. EAE was induced in recipient mice by the adoptive transfer of 25x10$^{6}$ MOG-reactive lymphocytes into IL-18Rα$^{-/-}$ and wt mice. Shown is one representative of 2 individual experiments ($n \geq 5$ mice/group).

**Figure 8**

IL-18Rα engagement promotes the production of IL-23p40. (a) ELISA of IL-12/23p40 production in the supernatant of T-cell depleted naive wt, IL-18$^{-/-}$ and IL-18Rα$^{-/-}$ splenocytes stimulated with 5 μg/ml αCD40 mAb for 36 hours. (b) ELISA of p40 expression in supernatant of LN cells from wt, IL-18$^{-/-}$ and IL-18Rα$^{-/-}$ mice immunized with $MOG_{35-55}$ in CFA, isolated 7 days later and restimulated with 50μg/ml $MOG_{35-55}$ or medium for 48 hours. (c) ELISA of p40 expression in supernatant of LN cells from wt, IL-18$^{-/-}$ and IL-18R$^{-/-}$ mice immunized with KLH in CFA, isolated 7 days later and restimulated with 50μg/ml KLH or medium for 48 hours. Shown is the mean ± S.E.M. Data are representative of 2 individual experiments combining at least 2 mice per group.

**Table 1**

Analysis of EAE onset and severity in wt and p35$^{-/-}$x IL-18$^{-/-}$ double-deficient mice. EAE was induced in wt and p35$^{-/-}$x IL-18$^{-/-}$ mice by subcutaneous immunization with $MOG_{35-55}$. Shown is disease incidence, mean day of onset and mean clinical score of diseased animals.

**Table 2**

Analysis of EAE onset and severity in wt, IL-18$^{-/-}$ and IL-18Rα$^{-/-}$ mice. EAE was induced in wt, IL-18$^{-/-}$ and IL-18Rα$^{-/-}$ mice by subcutaneous immunization with $MOG_{35-55}$. Shown is disease incidence, mean day of onset and mean clinical score of diseased animals.

**Supplementary Figure 1**

IL-18$^{-/-}$ LN cells do not produce IL-18. ELISA assessing IL-18 secretion by naïve wt and IL-18$^{-/-}$ LN cells, stimulated for 16 hours with the indicated stimuli of 1 μg/ml LPS, 100 U/ml IFN-γ, 5 μg/ml ConA and 2.5 ng/ml IL-12. Error bars represent ± S.E.M.

**Supplementary Figure 2**

CD4$^{+}$ T cells from IL-18$^{-/-}$ and IL-18Rα$^{-/-}$ mice are capable of IFN-γ production. CD4$^{+}$ T were purified from naïve wt, IL-18$^{-/-}$ and IL-18Rα$^{-/-}$ splenocytes by magnetic cell sorting and were stimulated with αCD3 mAb or a combination of αCD3 and αCD28 mAbs for 36 hours *in vitro*. IFN-γ secretion by stimulated cells was determined by ELISA. Error bars represent ± S.E.M. Data are representative of at least 2 individual experiments.

**Supplementary Figure 3**

Analysis of inflammatory infiltrates, cytokines and chemokines in MOG$_{35-55}$-immunized mice. (a) Quantification of CD3$^{+}$, MAC3$^{+}$, B220$^{+}$ and APP$^{+}$ cells in histological sections of spinal cord from actively immunized wt (n=8), IL-18$^{-/-}$ (n=5) and IL-18Rα$^{-/-}$ (n=4) mice 28 days post-immunization. (b) Real-time PCR analysis of chemokine and inflammatory cytokine mRNA expression in the spinal cords of MOG$_{35-55}$-immunized wt, IL-18$^{-/-}$ and IL-18Rα$^{-/-}$ mice 14 days post-immunization. (c) Quantification of CNS-invading leukocytes of MOG$_{35-55}$-immunized wt, IL-18$^{-/-}$ and IL-18Rα$^{-/-}$ mice 7 days post-immunization, pre-disease onset. Leukocytes were isolated from the CNS of mice using Percoll and were stained with αCD45, αCD11b, αGR1, αB220 and αCD4 Abs to assess the percentage of macrophages, granulocytes, B cells and CD4+ T cells infiltrating the CNS by flow cytometry.

**Supplementary Figure 4**

Intracellular staining for IL-17 and IFN-γ. Mice were injected with $25x10^6$ OVA-specific TcR Tg OT-II splenocytes i.v. and immunized with 200μg OVA protein. 5 days later, lymphocytes were isolated and restimulated with 50 ng/ml PMA and 500 ng/ml ionomycin for 5 hours. Numbers in quadrants indicate the percent of cells staining positive for the indicated cytokines.

**Supplementary Table 1**

α-IL-18Rα Ab does not affect the composition of the peripheral immune compartment. IL-$18^{-/-}$ mice were subcutaneously immunized with $MOG_{35-55}$ and treated with anti-IL-18Rα Ab or IgG. The composition of leukocytes in LNs, spleen and blood was analyzed 7 days later by flow cytometry. Shown is the percentage of leukocytes and accessory cells in LN cells, which is representative of spleen and blood.

Figure 1

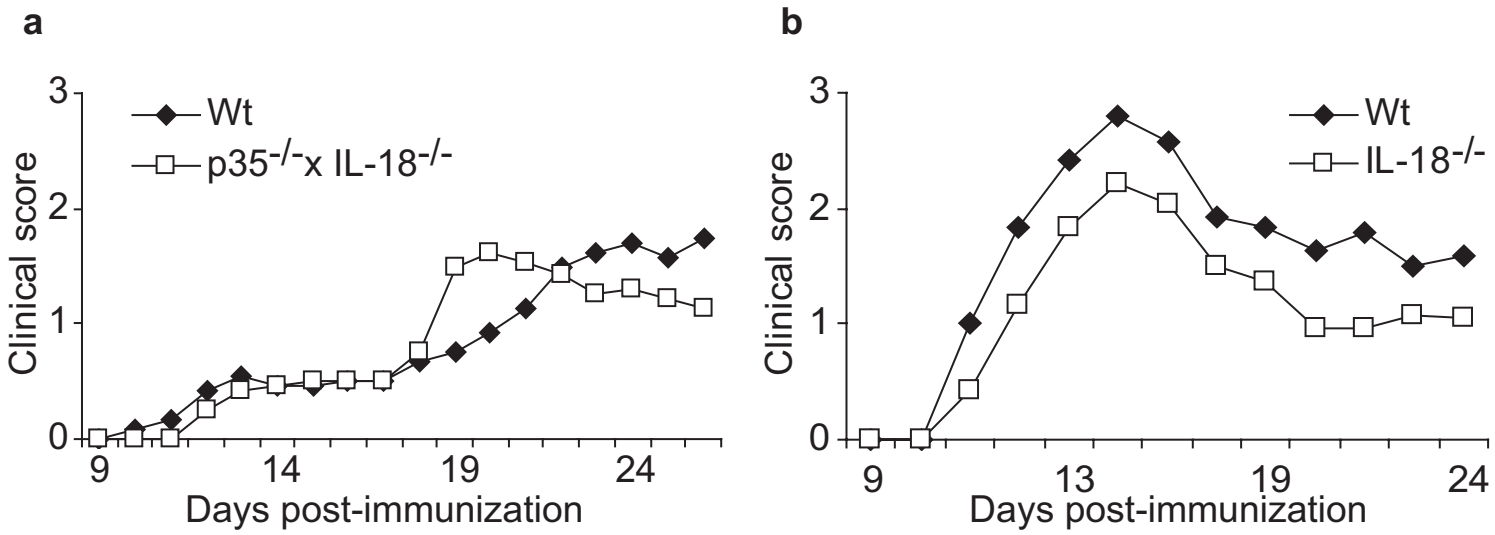

a
b
Clinical score
Days post-immunization
Wt
p35-/-x IL-18-/-
IL-18-/-
0
1
2
3
9
13
14
19
24

Table 1

p35$^{-/-}$x IL-18$^{-/-}$ mice are susceptible to active EAE

| **Mouse genotypes** | **Incidence (%)** | **Mean day of disease onset** | **Mean maximal clinical score (+/- SEM)*** |
|---|---|---|---|
| Wt | 8/10 (80) | 14.2 | 2.4 +/- 0.25 |
| p35$^{-/-}$x IL-18$^{-/-}$ | 7/10 (70) | 14.1 | 3.2 +/- 0.08 |

* of diseased animals

Figure 2

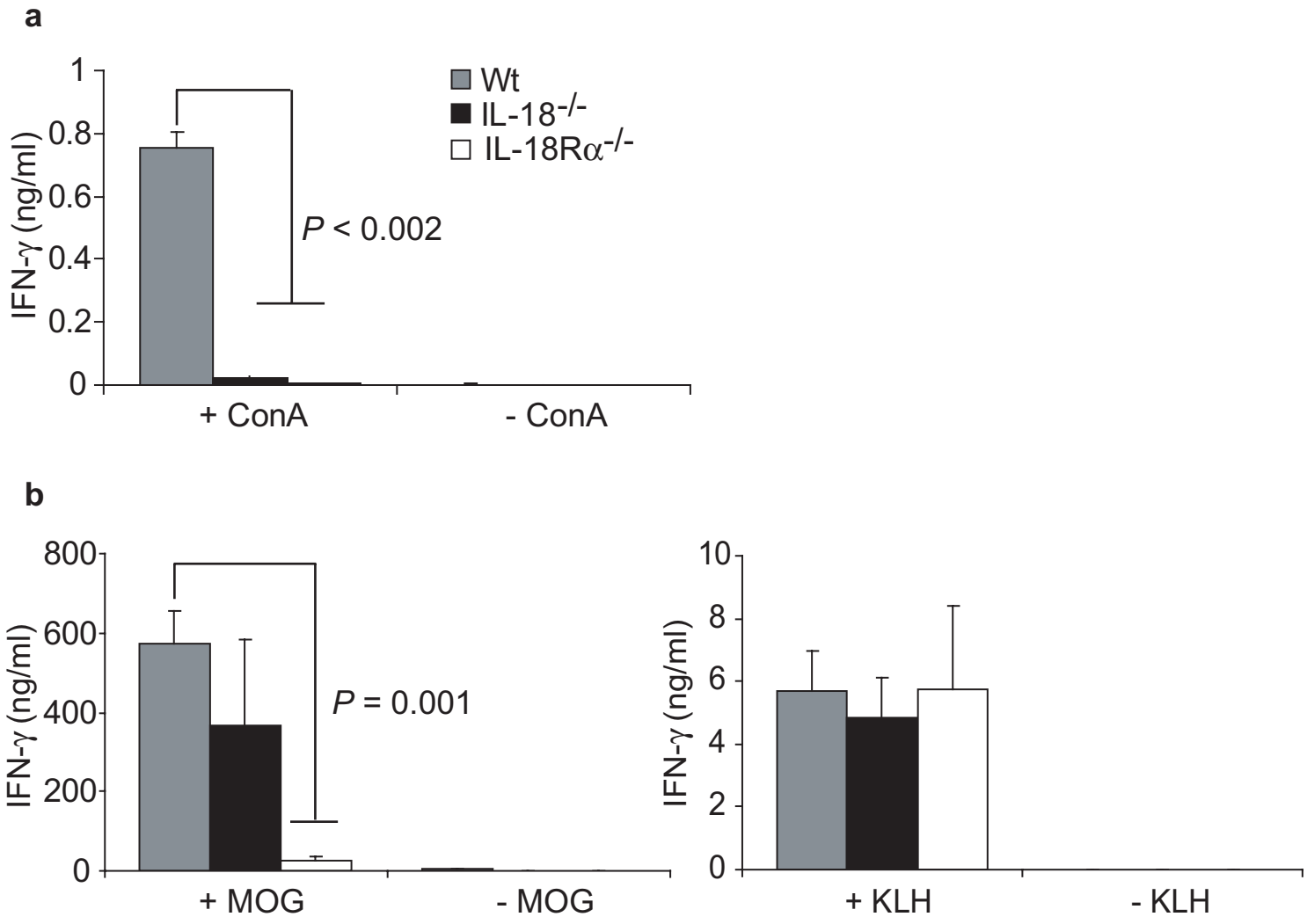

a
IFN-γ (ng/ml)
1
0.8
0.6
0.4
0.2
0
Wt
IL-18-/-
IL-18Rα-/-
P < 0.002
+ ConA
- ConA
b
IFN-γ (ng/ml)
800
600
400
200
0
P = 0.001
+ MOG
- MOG
IFN-γ (ng/ml)
10
8
6
4
2
0
+ KLH
- KLH

Table 2

IL-18Rα is critical for the development of active EAE in mice

| **Mouse genotypes** | **Incidence (%)** | **Mean day of disease onset** | **Mean maximal clinical score (+/- SEM)*** |
|---|---|---|---|
| Wt | 17/20 (85) | 11.8 | 2.6 +/- 0.13 |
| IL-18$^{-/-}$ | 20/22 (91) | 12.8 | 2.35 +/- 0.13 |
| IL-18Rα$^{-/-}$ | 2/20 (10) | 18.5 | 2.6 +/- 0.12 |

* of diseased animals

Figure 3

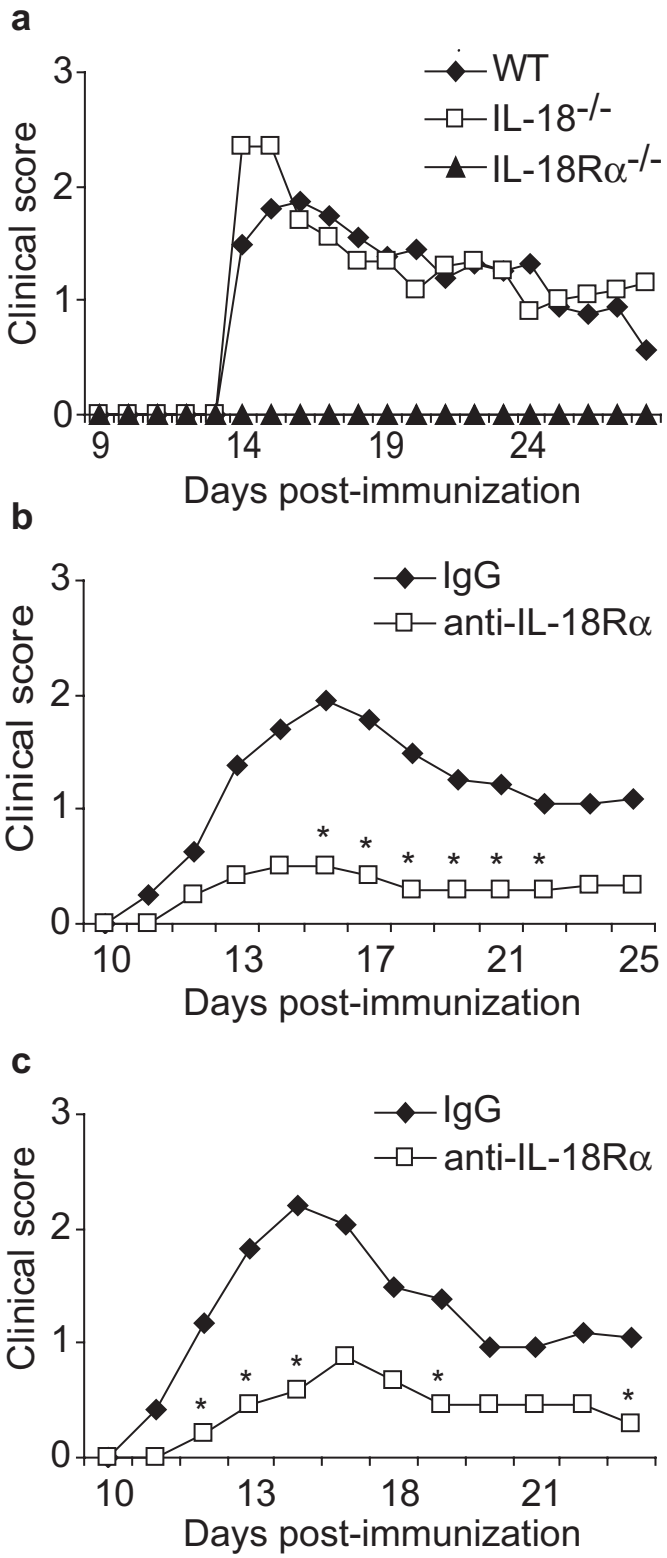

a
WT
IL-18-/-
IL-18Rα-/-
Clinical score
Days post-immunization
b
IgG
anti-IL-18Rα
Clinical score
Days post-immunization
c
IgG
anti-IL-18Rα
Clinical score
Days post-immunization

Figure 4

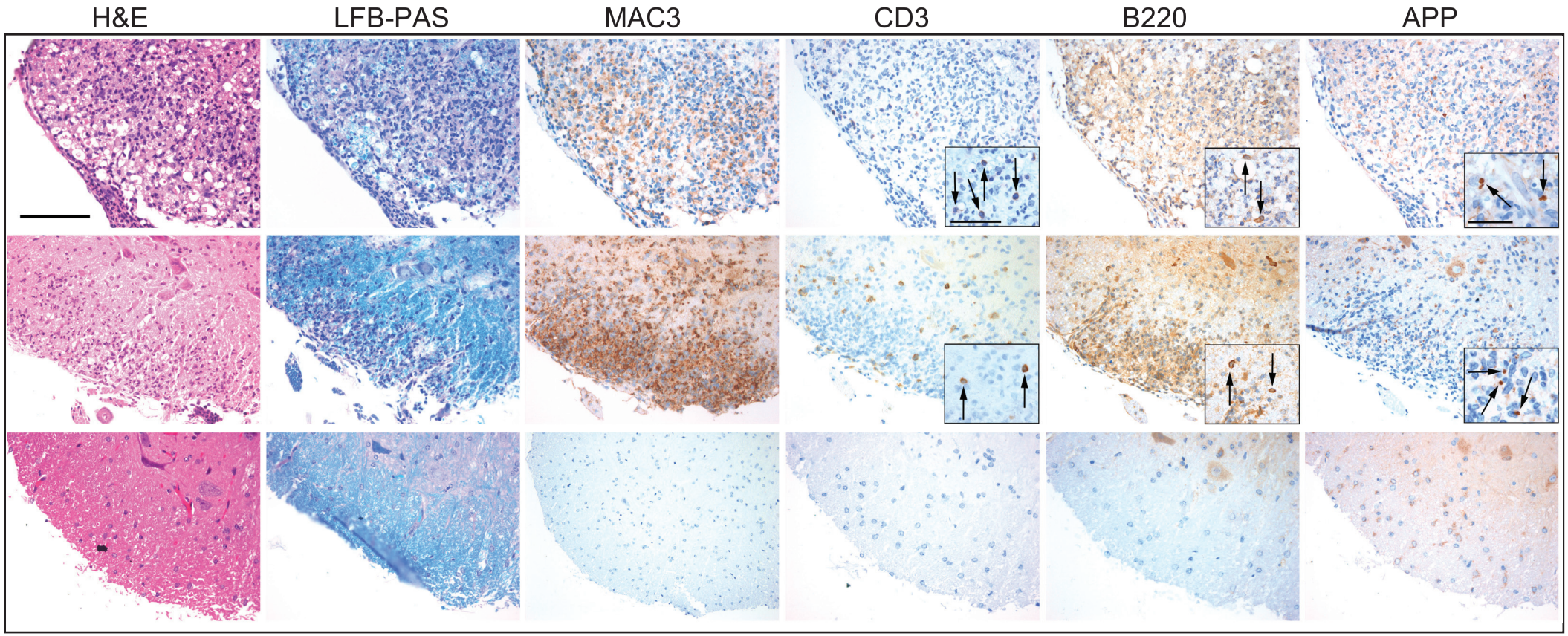


Figure 5

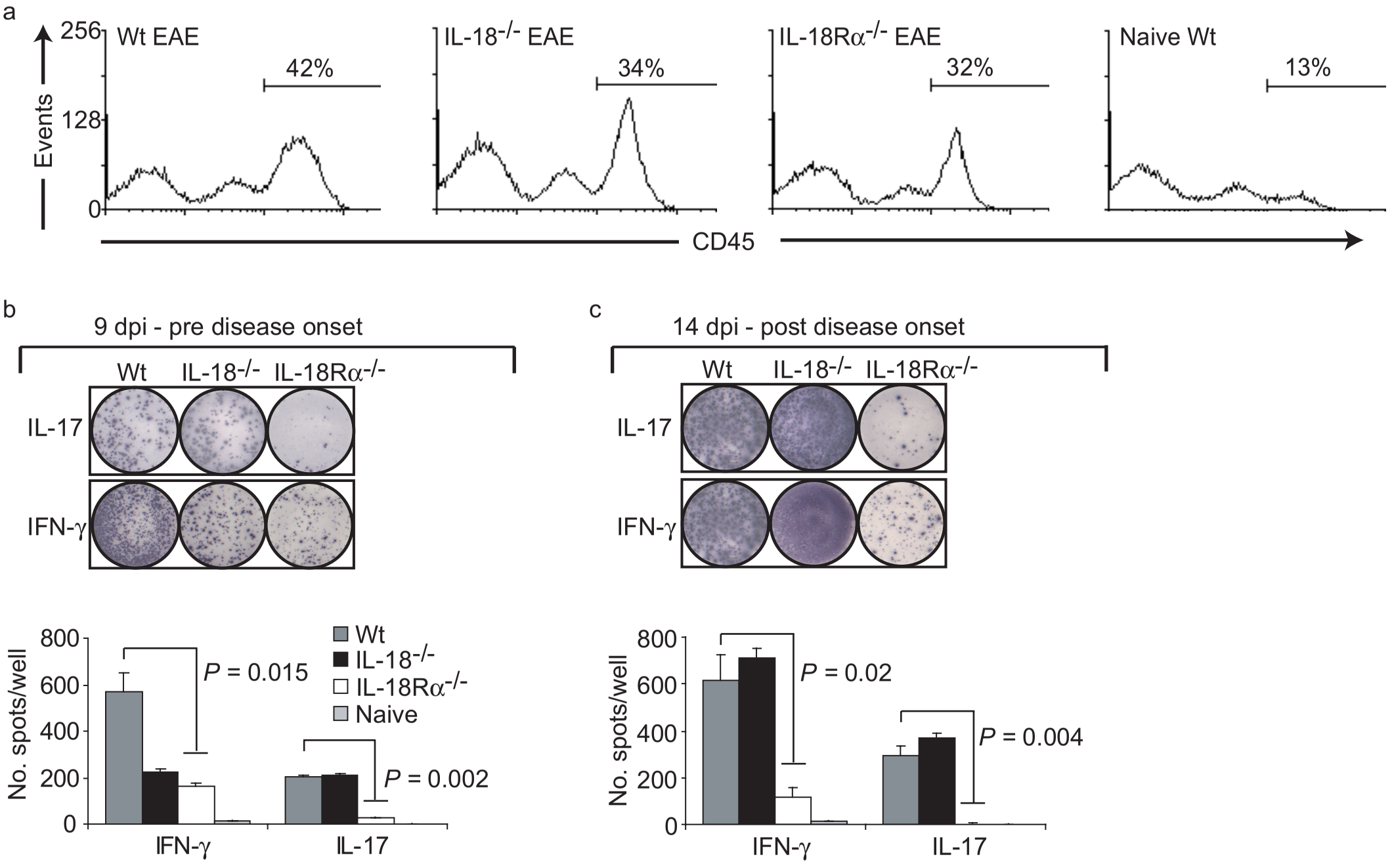

a
256
128
0
Events
Wt EAE
42%
IL-18-/- EAE
34%
IL-18Rα-/- EAE
32%
Naive Wt
13%
CD45
b
9 dpi - pre disease onset
Wt
IL-18-/-
IL-18Rα-/-
IL-17
IFN-γ
800
600
400
200
0
No. spots/well
P = 0.015
P = 0.002
Wt
IL-18-/-
IL-18Rα-/-
Naive
IFN-γ
IL-17
c
14 dpi - post disease onset
Wt
IL-18-/-
IL-18Rα-/-
IL-17
IFN-γ
800
600
400
200
0
No. spots/well
P = 0.02
P = 0.004
IFN-γ
IL-17

Figure 6

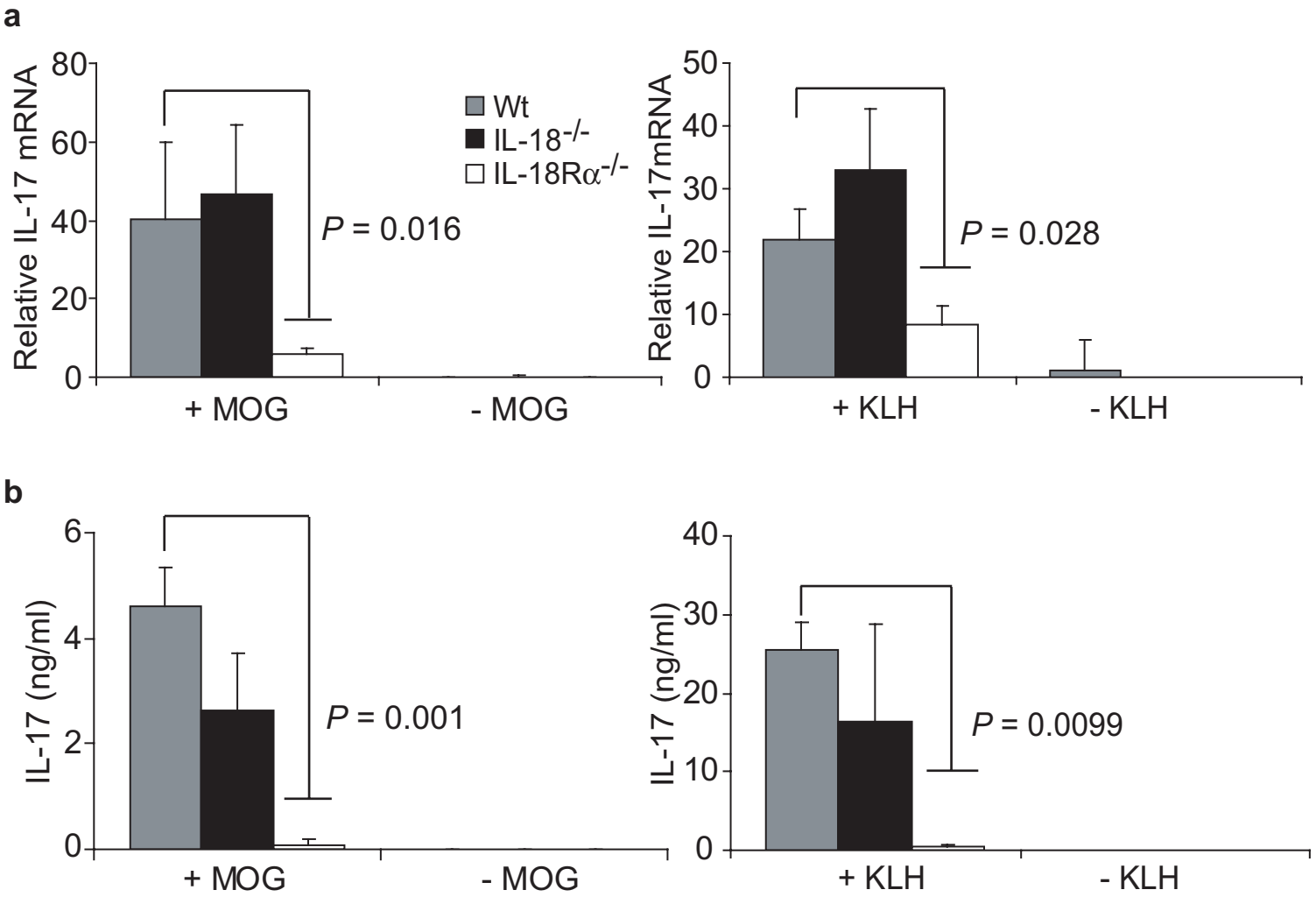

a
Relative IL-17 mRNA
80
60
40
20
0
Wt
IL-18-/-
IL-18Rα-/-
P = 0.016
+ MOG
- MOG
Relative IL-17mRNA
50
40
30
20
10
0
P = 0.028
+ KLH
- KLH
b
IL-17 (ng/ml)
6
4
2
0
P = 0.001
+ MOG
- MOG
IL-17 (ng/ml)
40
30
20
10
0
P = 0.0099
+ KLH
- KLH

Figure 7

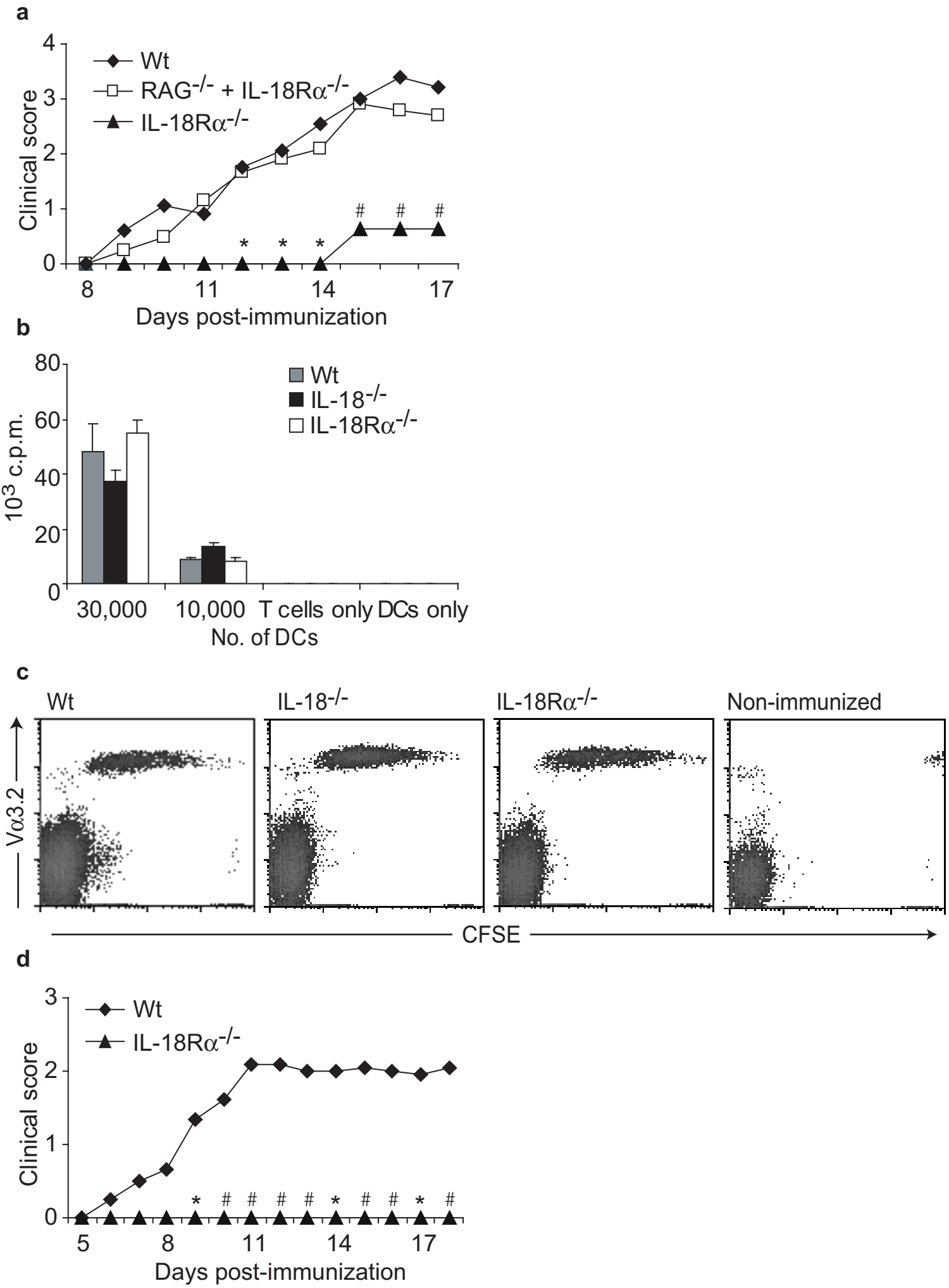
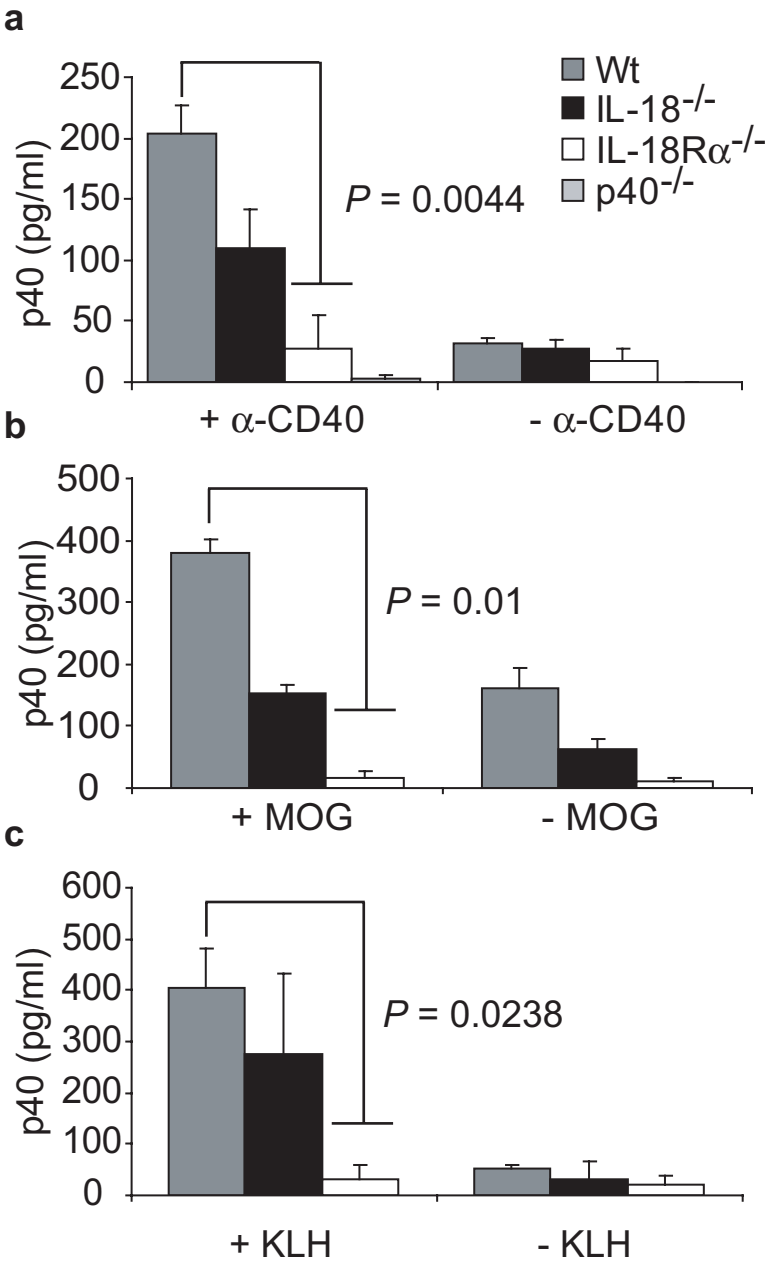

a
Wt
RAG-/- + IL-18Rα-/-
IL-18Rα-/-
Clinical score
Days post-immunization
b
Wt
IL-18-/-
IL-18Rα-/-
10³ c.p.m.
30,000
10,000
T cells only
DCs only
No. of DCs
c
Wt
IL-18-/-
IL-18Rα-/-
Non-immunized
Vα3.2
CFSE
d
Wt
IL-18Rα-/-
Clinical score
Days post-immunization

Figure 8


a
250
200
150
100
50
0
p40 (pg/ml)
Wt
IL-18-/-
IL-18Rα-/-
p40-/-
P = 0.0044
+ α-CD40
- α-CD40
b
500
400
300
200
100
0
p40 (pg/ml)
P = 0.01
+ MOG
- MOG
c
600
500
400
300
200
100
0
p40 (pg/ml)
P = 0.0238
+ KLH
- KLH